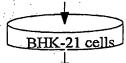
Isolation of Non-Cytopathic (NCP) VSV

Mutants

Infect cells with rVSV-M33,51A-GFP



After 24 hrs wash monolayer to remove cells exhibiting CPE (rounded and floating)

Maintain culture in incubator

but that lack CPE

Select for non-cytopathic variants by screening for adherent GFP (+) cells

Harvest supernatant & Infect fresh cells

BHK-21 cells

Identify cultures that are GFP (+),

Collect supernatant and titer

Obtain plaque isolates, generate stocks and characterize virus growth properties, and cell rounding phenotypes

Figure 1

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Cell Rounding Phenotypes of M Protein Mutants of VSV

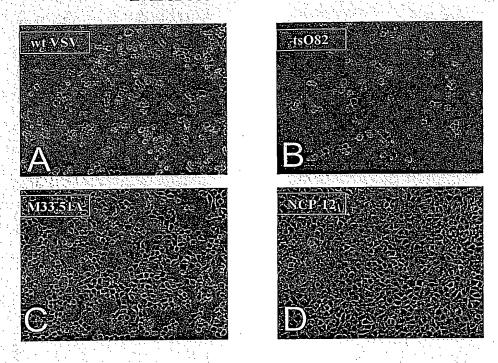


FIGURE 2

Isolation and Sequencing of NCP-M Protein Mutant cDNAs

Purify NCP virus from culture supernatants of plaque purified NCP isolates

Isolate genomic RNA and perform RT-PCR using M gene specific primers

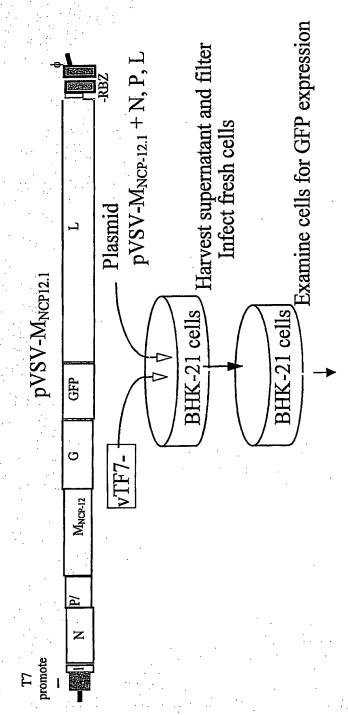
Subclone cDNAs into plasmid pBS-SK+

Sequence individual clones and identify mutations present in M_{NCP} cDNAs

NCP mutations include the original M-A changes and two additional C-terminal mutations (Designated M_{NCP12.1})

Subclone M_{NCP12.1} into pVSV-FL(+)-2

Recovery of rVSV-IMINCP12.1



Collect supernatant and titer

btain plaque isolates, generate stocks and characterize growth properties, and cell rounding

Figure 4

rVSV/NCP-12.1 is Defective in Cell Rounding

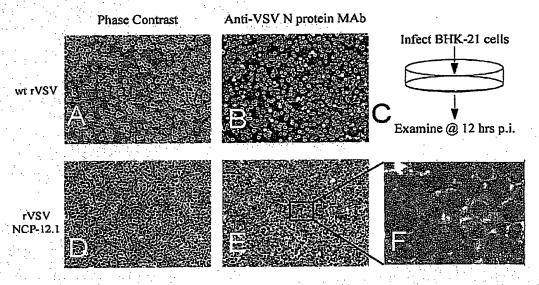
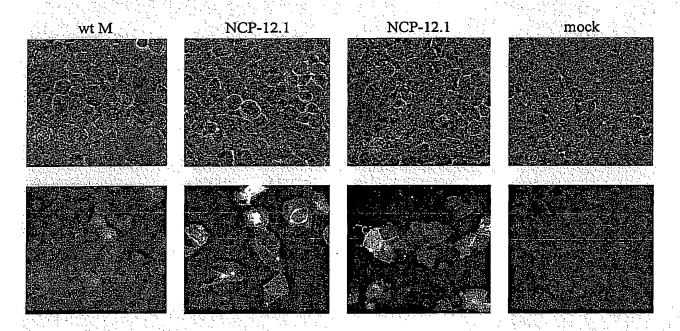


Figure 5

Transient Expression of $M_{NCP-12.1}$



rVSV/M_{NCP-12.1} Infection of Different Cell Types

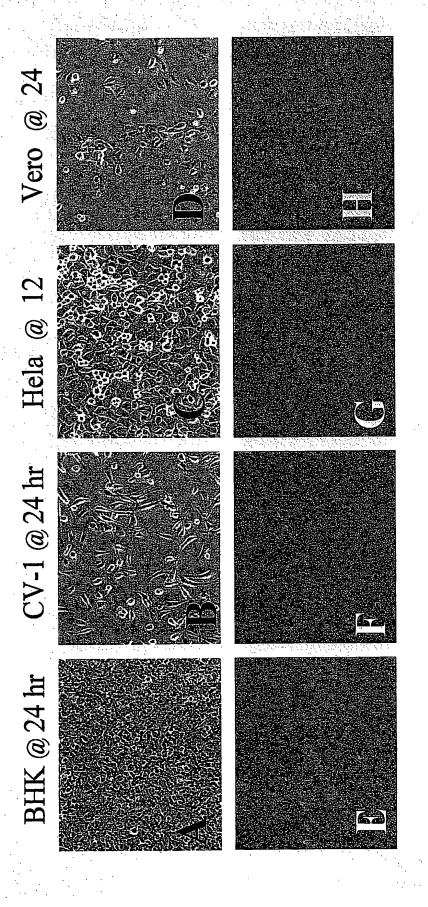
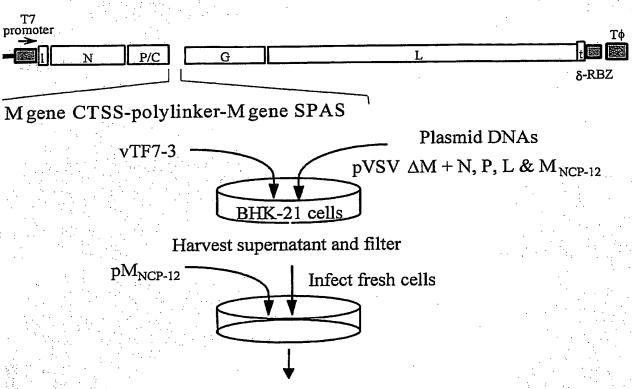


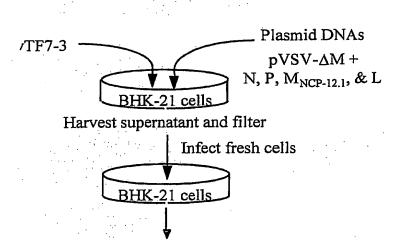
Figure 7

Use of $M_{NCP-12.1}$ to Recover ΔM -VSV

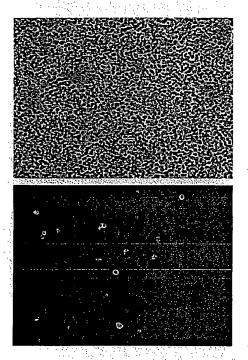


Collect supernatant and examine for N(+) cells

Recovery of rVSV- ΔM



Examine for N protein expression



Sample #176, 3 Days Post-infection

Infection of Islet Prep#176 at day 3 post infection

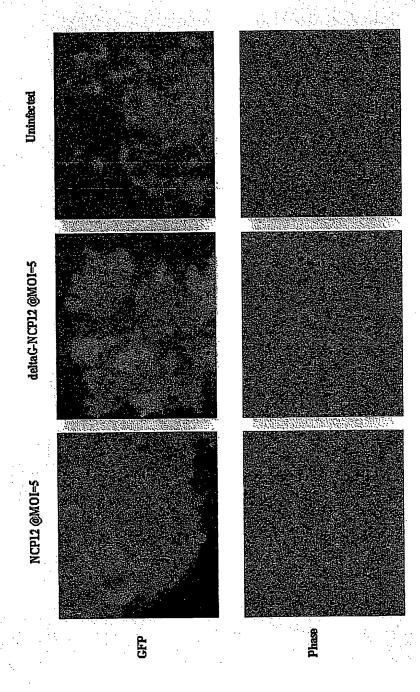


Figure 10

Sample #163, 3 Days Post-infection

Infection of islet Prep#163 at day 3 post infection

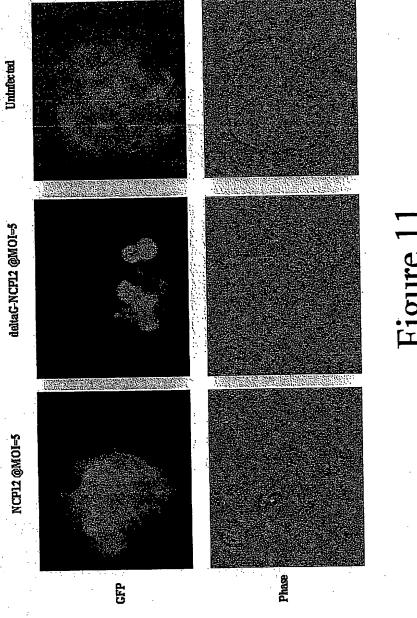


Figure 11

Sample #176, 3 Days Post-infection

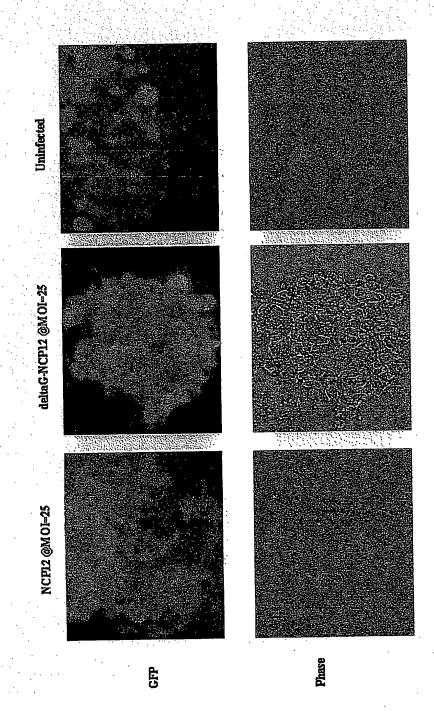


Figure 12

Sample #163, 3 Days Post-infection

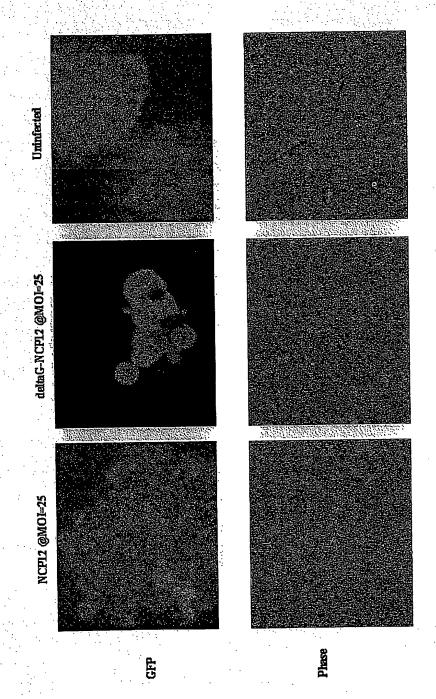


Figure 13

Sample #176, 8 Days Post-infection

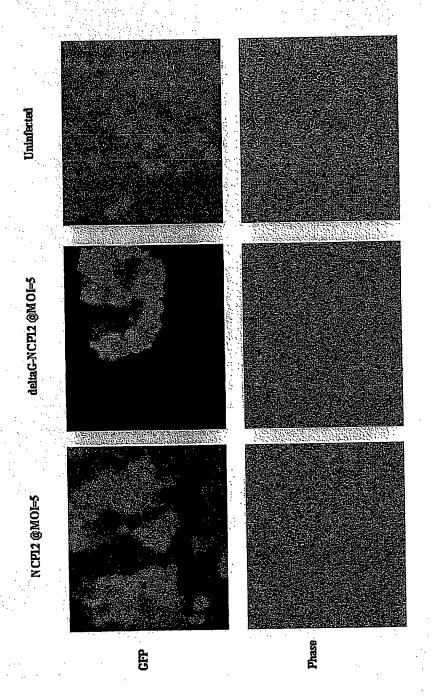


Figure 14

Sample #176, 8 Days Post-infection

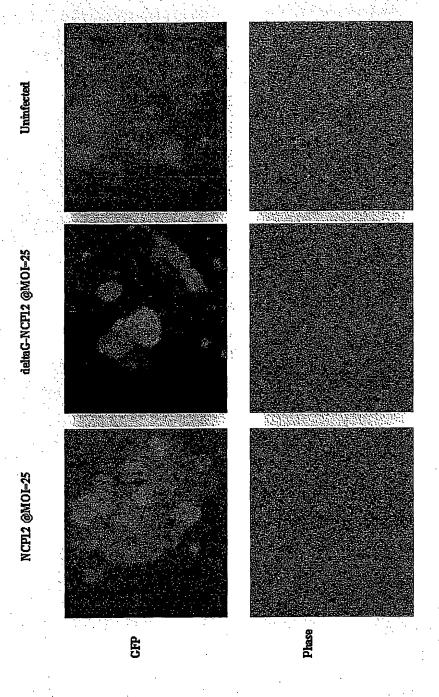


Figure 15

Sample #163, 8 Days Post-infection

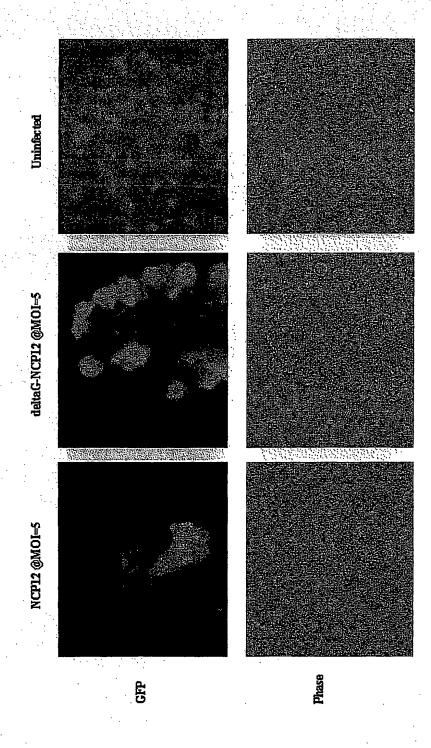


Figure 16

Sample #163, 8 Days Post-infecti

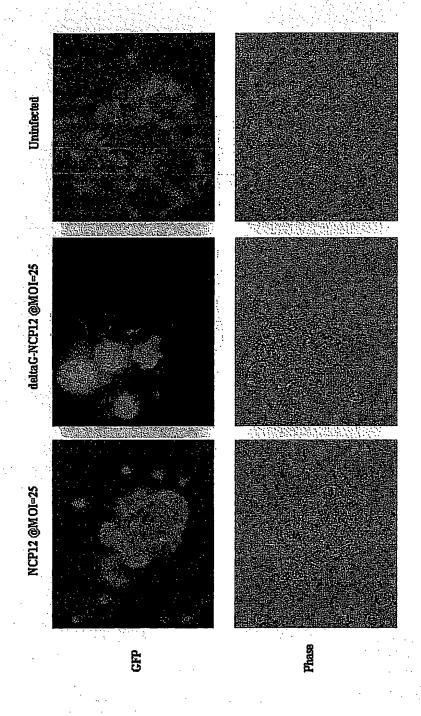


Figure 17

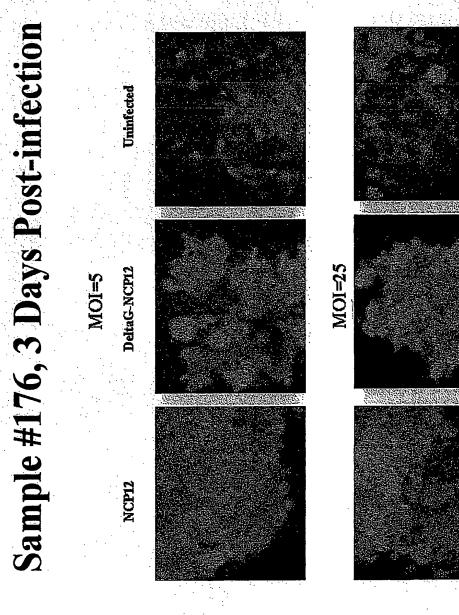


Figure 18

Sample #176, 8 Days Post-infection

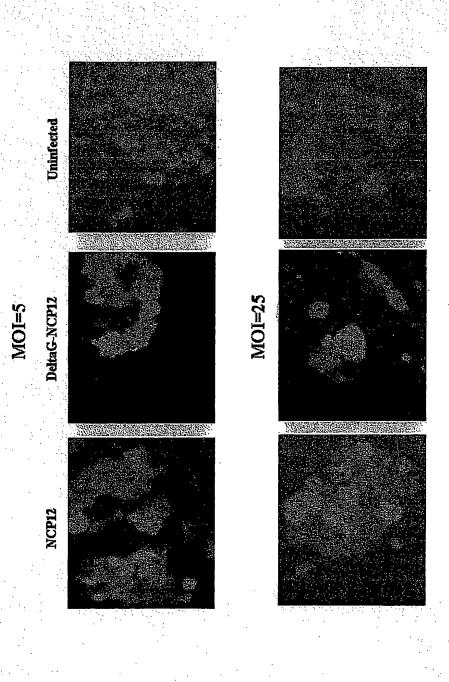


Figure 19

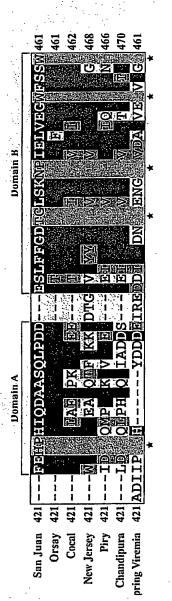


figure so

1421FEHPHIQDAASQLPDDESLFFGDTGLSKNPIELVEGWFSSWK4621

\mathbf{A} .		E452A(E-A)		
D		G456D(G-D)		
A		W457A(W1A)		
A		F458A(F-A)		
	A	W461A(W2A)		
A	A	W457A,W461A(WW-AA)		
DA		G456D,W457A(DA)		
AA	A	W457A, F458A, W461A (AAA)		
DA	A	G456D,W457A,W461A(DAA)		

FEHPHIQDAASQLPDDESLFFGDTGLSKNPWSSFWGEVLEIK

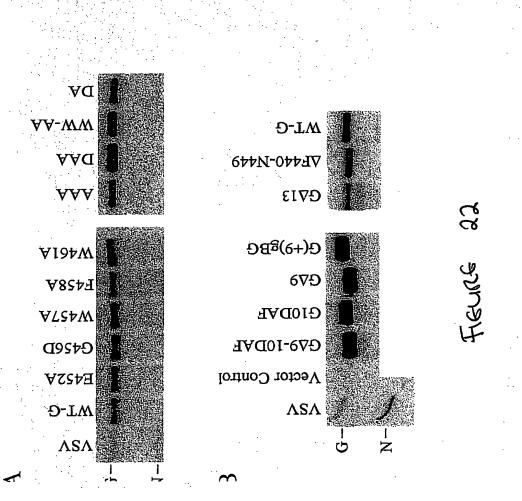
FEHPHIQDAASQLPDDESLFFGDTGLSKNPASSFAGEVLEIK

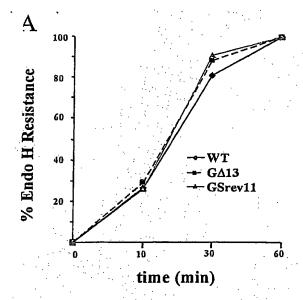
FEHPHIQDAASQLPDDESL------PIELVEGWFSSWK

FEHPHIQDAASQLPDDESLFFGDTGLSKNPIELVEGWFSSWK

FEHPHIQDAASQLPDDESLFFGDTGLSKNPIELVEGWFSSWKS

FEHPHIQDAASQLPDDESLFFGDTGLSKNPIELVEGWFSSWKSS





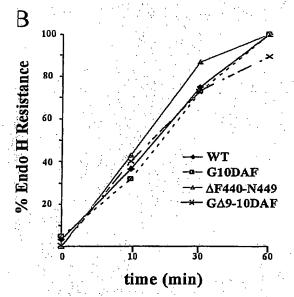
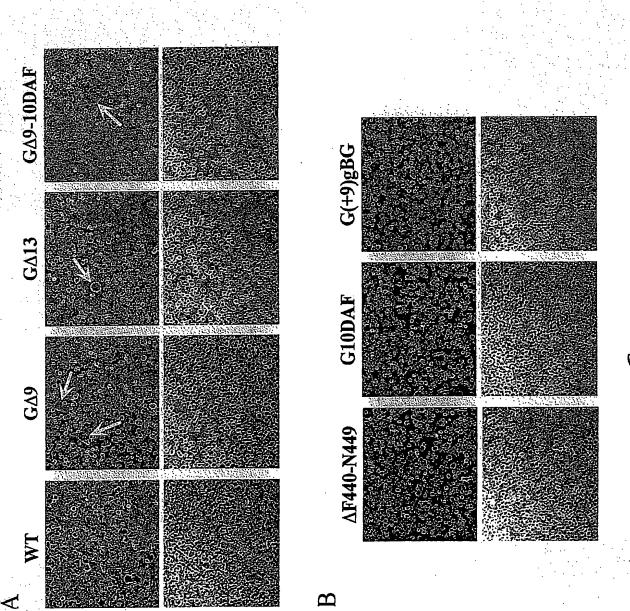
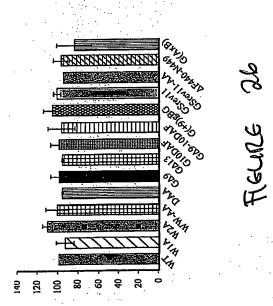


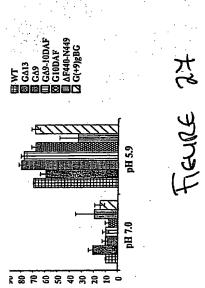
FIGURE 23

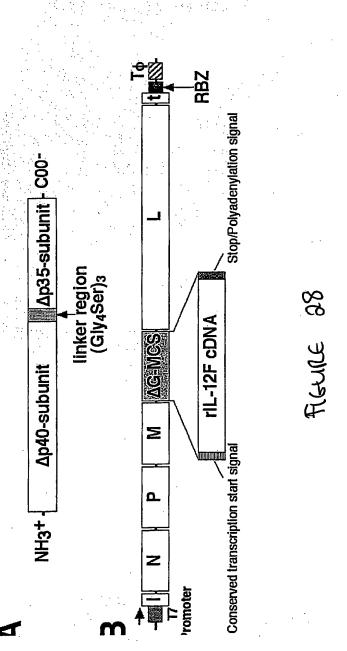


Fleurice 24

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DE+10 JE+09 JE+08 JE+06 JE+05 JE+04 JE+03 JE+03 JE+03	15400 L		25	旅養衛	欧金田
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1E+10 1E+09 1E+08 1E+07 1E+06 1E+05					西
DE+10 DE+09 DE+08 DE+07 DE+06 DE+05	JE+04		SAC D	10000000	12000000
E+10 1E+09 1E+08 1E+07 1E+07 1E+06				RESEAR L	推翻器
DE+10 DE+09 DE+08 DE+07 DE+07	1E+05	- 1888 E	200	200	2000 T
1E+10 1E+09 1E+08 1E+07 1E+06	1	1000000	POST STATE	150	一种特殊
E+10 1E+09 1E+08 1E+07	*A17+00 -	200			经经验
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E+10 E+09 E+08					
E+10 T	E700	14年新9	100 A		(सम्बद्धितः)
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			120 E	ल्बॉन्स	
	E+10				
E+11			(Sept. 1962)		
	1E+11				···
E+12	I				







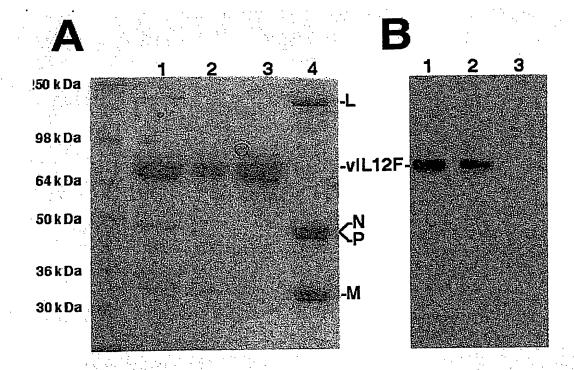
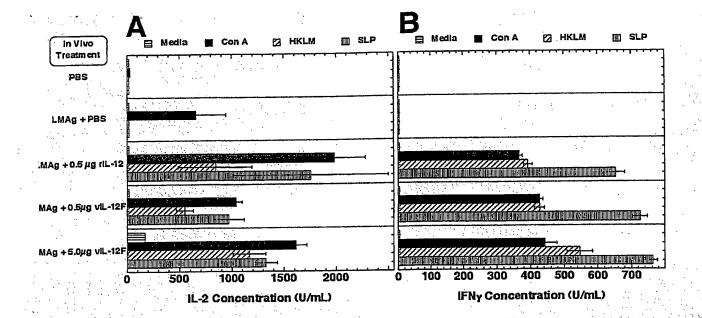


FIGURE 29



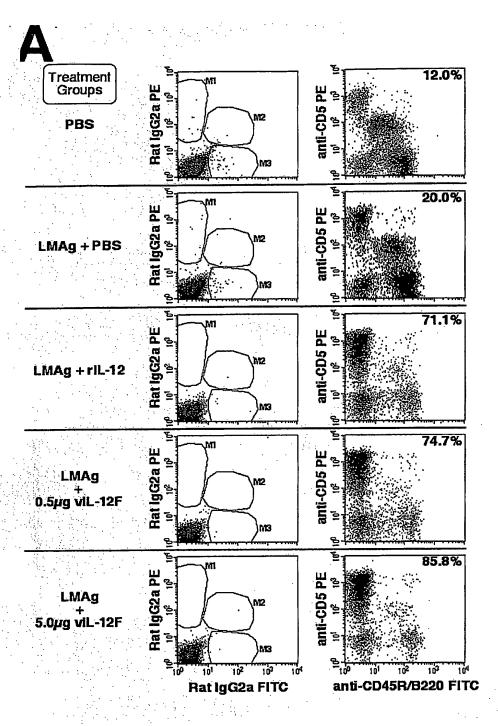
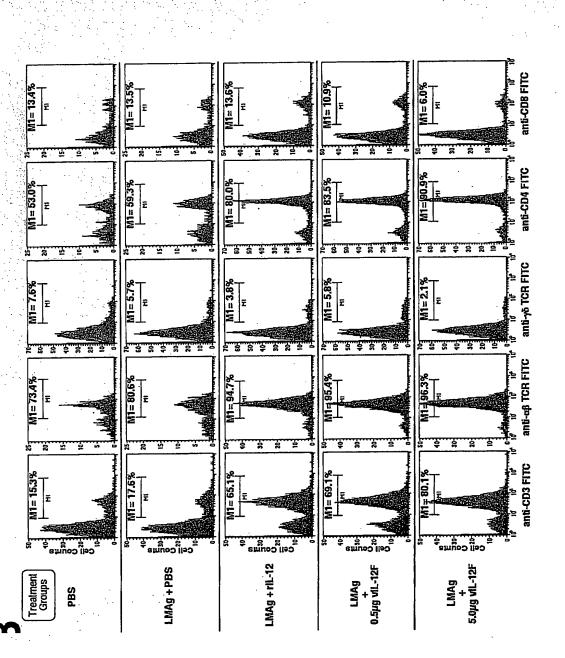


FIGURE 3/9



Fleure 31 p

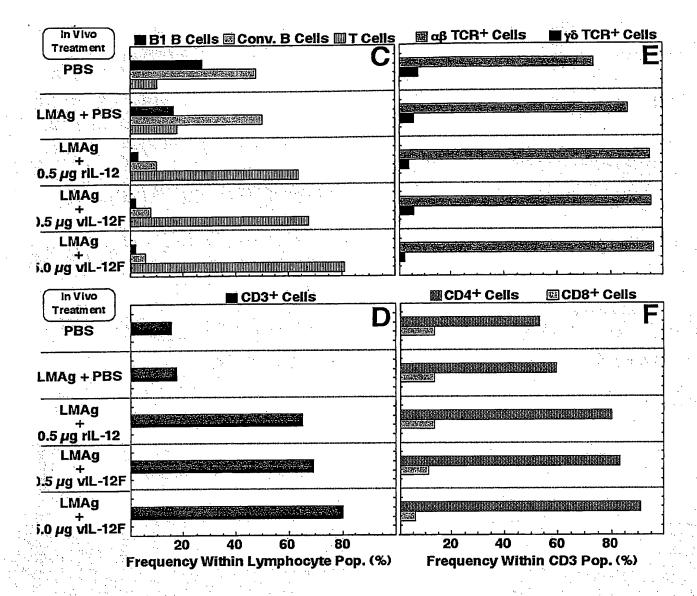
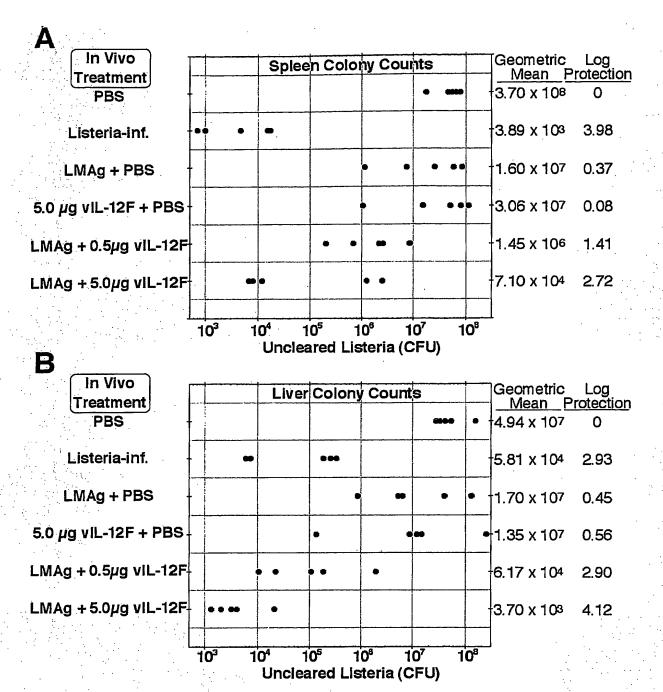


FIGURE 31 C



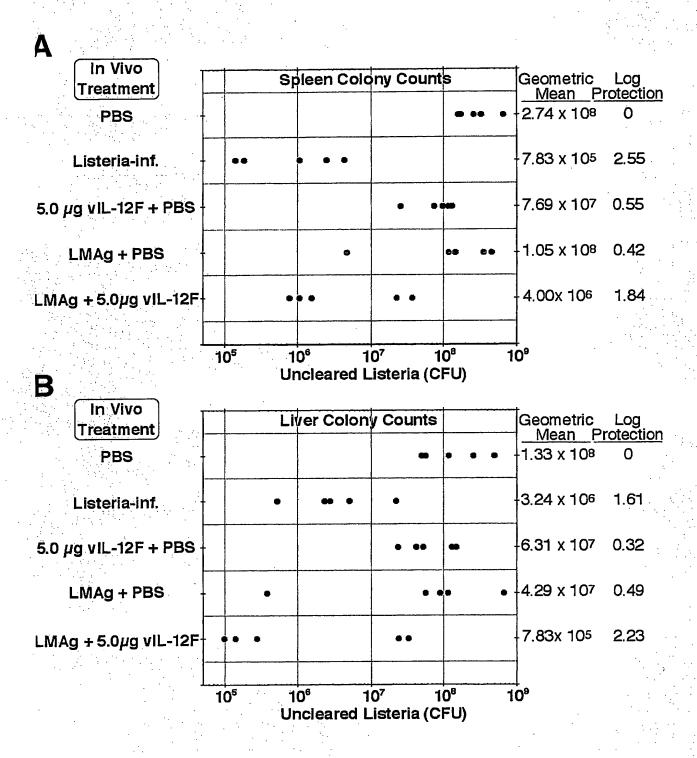


FIGURE 33

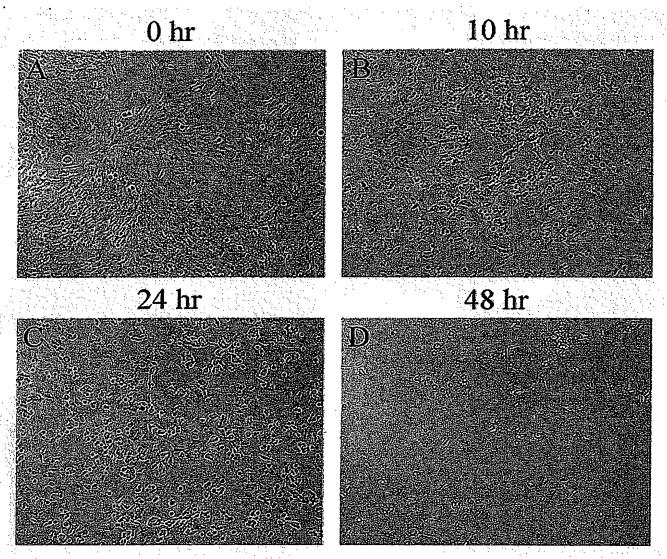


Figure 34

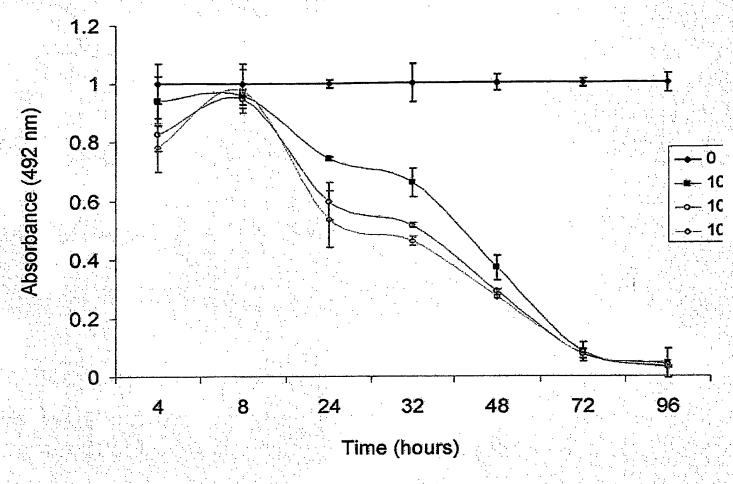


Figure 35

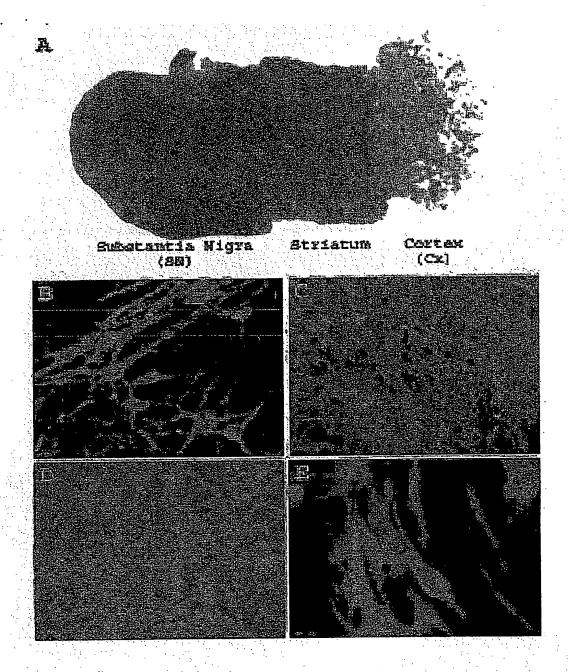


Figure 36

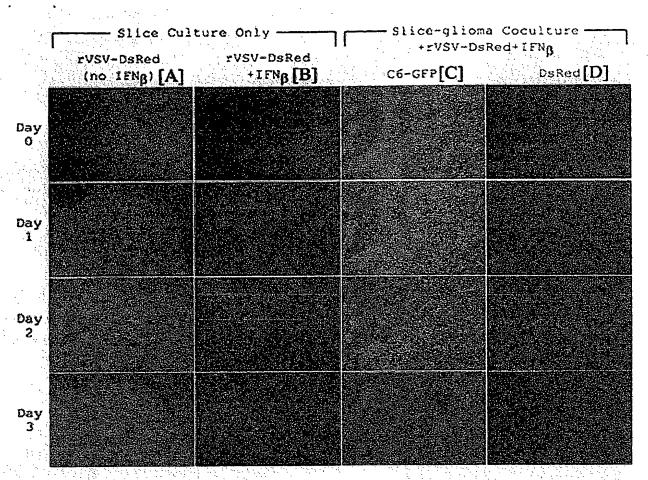


Figure 37

VSV-wt w/ 1,000u IFN on C6 co-cx

normal slice w/ IFN only

Figure 38

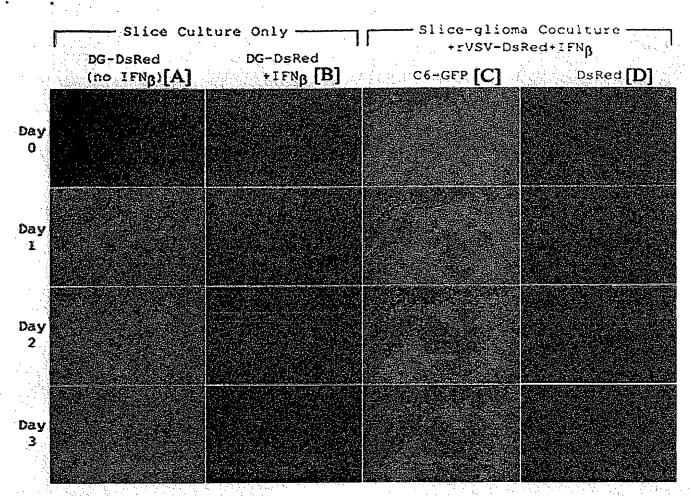


Figure 39

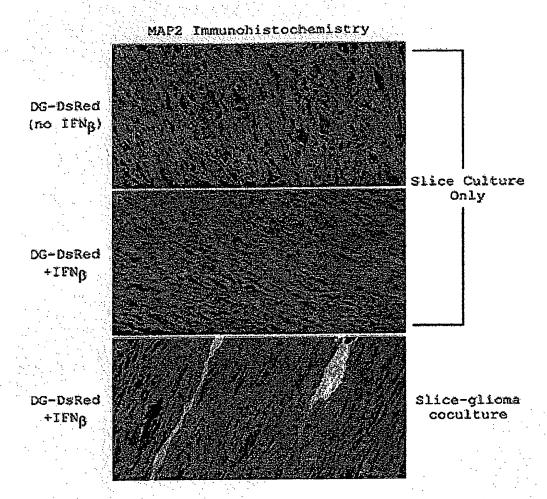


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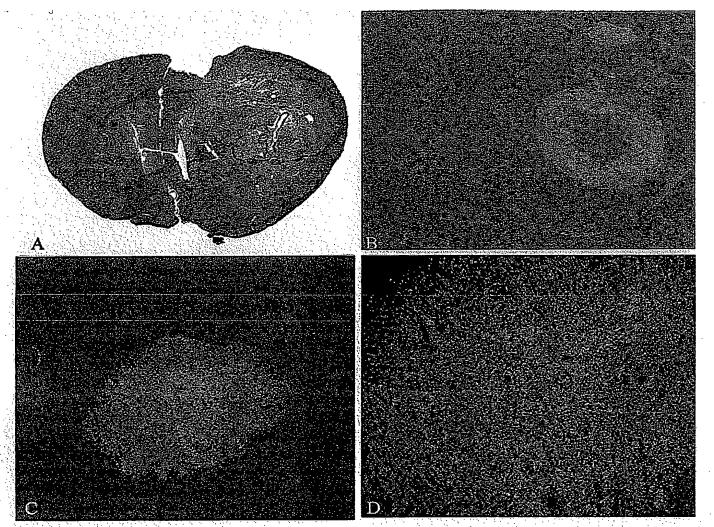


Figure 41

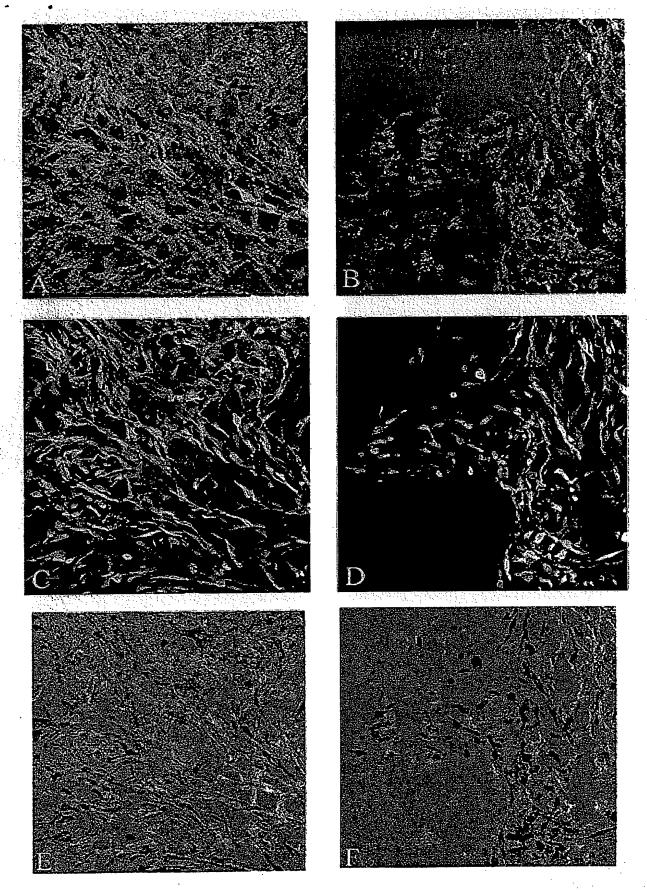


Figure 42

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